RESEARCHES ON TETANUS

II. The Toxin of the Bacillus tetani Is Not Transported to the Central Nervous System by Any Component of the Peripheral Nerve Trunks

BY

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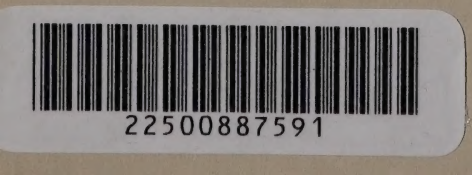

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RESEARCHES ON TETANUS

II. THE TOXIN OF THE BACILLUS TETANI IS NOT TRANSPORTED TO THE CENTRAL NERVOUS SYSTEM BY ANY COMPONENT OF THE PERIPHERAL NERVE TRUNKS[1,2]

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In the years 1884 to 1890, as is well known, bacteriologists definitely established the infective nature of the fatal disease, tetanus, by isolating the causative organism in pure culture. One ordinarily speaks of this organism as the *bacillus tetani*, but it is known to the specialist as *clostridium tetani*, one of the family of the bacillaceae of the general class of plant organisms known as Schizomycetes. That the disease may appear, this clostridium or its spores must be introduced beneath the skin. The history of tetanus shows that there are innumerable ways in which the organism may gain entrance into the body. Thus, it may occur during or after the application of blistering plasters, cupping, extraction of teeth, the application of ligatures to the umbilical cord and to wounds, after slight lesions of any part of the integument, even of the cornea, stings and bites of insects, in the course of gangrene, after scratching acne pustules, after compound fractures and other extensive injuries such as gun-shot and knife wounds, and so

[1] A grant from the Carnegie Corporation of New York has made it possible for the authors to carry on a series of investigations on tetanus.

[2] The senior author has given in an earlier communication a brief account of Professor Lee's experiments on the transection and suture of sciatic nerves into which tetanus toxin was later injected, and also an abstract of our own determinations of the amount of toxin in the blood and lymph of sheep throughout the course of experimentally induced tetanus. The communication appeared in Science, 1934, vol. lxxix, pp. 121–129, and will henceforth be called No. I of our series of papers on tetanus.

forth; in fact, after every kind of lesion of the body surface or of its orifices. At any point of its entrance into the body the bacillus readily multiplies if it meets with suitable conditions for its growth, such as association with relatively harmless pus-forming organisms, as is generally the case, and a diminished tension of oxygen. If washed spores of the bacillus are introduced beneath the skin, their development will take place only if a necrosis of tissue cells is at the same time produced by one method or another. It is well known, too, that the bacilli are not dispersed throughout the body but continue to multiply for a time at the site of inoculation. Only very rarely and under special circumstances are the organisms or their spores found elsewhere in the body. During the time of bacillary growth in the infected area a highly poisonous substance called tetanus toxin appears. This exceedingly potent, water-soluble substance has been definitely proved to be the true cause of the disease. A few bacteriologists believe that the bacillus does not itself produce a toxin but rather a relatively harmless precursor or pro-toxin which is transformed into the true toxin in culture media or at the site of infection in the living animal in the presence of albumose-like substances in the cultures or in the tissues of the infected animal. Nothing whatever is known with certainty of the true chemical nature of the toxin. We cannot even be certain that only one tetanizing poison is produced by the bacilli, but the probabilities are greatly in favor of the supposition that they elaborate a single highly toxic substance. This is being constantly removed from its place of origin by lymphatic vessels and by the blood capillaries, to be then distributed throughout the body by the arterial current. It is evident that we are dealing in the case of this disease with a form of poisoning by a chemical agent. A person with lockjaw, or tetanus, is as truly poisoned as if he had been bitten by a venomous serpent.

According to the generally accepted theory of our day, the disease is defined as being clearly and solely one of the central nervous system; and therefore, in accordance with the prevailing theory, *none* of its symptoms can appear until *after* the toxin has reached certain cells of the spinal cord, medulla or pons. Certain of the symptoms of the disease, however, such as the so-called "local tetanus" and the long-continued rigid and unremitting contractures of certain muscles,

lasting for months or even years, that have been observed in human beings and for shorter periods in experimental animals, cannot easily be brought into line with this concise definition of the disease.

LOCAL TETANUS

It is known to every experimenter on tetanus that if one injects a small quantity of the poison into an extremity all of its muscles will, after an interval of time varying from twenty-four hours or so to four days or more, become so rigid that all freedom of movement disappears. The leg is now stiffly extended and so useless that the animal walks on three legs. This condition may exist for weeks without the appearance of a *trace of reflex excitability or any other symptom* of the disease, when only a very small dose of the toxin has been injected. It is to such a condition that the term local tetanus is well applied, but even a single muscle can be thrown into this state of contracture by the injection into it of a very small dose of the toxin. The quantity of toxin that is required to stiffen *all* of the muscles of one hind leg of a dog is less than 1/200 of the usually accepted lethal dose for this animal, as will be shown in a later communication from our laboratory. If the injected dose in the instance cited is very small the local tetanus finally disappears and the animal regains its normal state.

Hundreds of instances of local tetanus involving only certain groups of muscles or even an entire extremity are to be found in the case histories of human beings with tetanus. In these clinical cases, as in animal experiments, the condition may exist for a variable period of time without a trace of reflex excitability. Local tetanus is so very frequently overlooked by the physician, either because he underestimates its theoretical significance, or because by the time the patients reach his hands they already present the more arresting symptoms of the disease, such as difficulty in swallowing, trismus, stiffening of the muscles of the neck and back, or even convulsive seizures of more or less generalized character. Local tetanus must inevitably make its appearance in those muscles of a human being, as in those of a laboratory animal, that happen to lie in the immediate neighborhood of the area in which the multiplying clostridia are producing their toxin.

There is one type of the human disease in which local tetanus is

perhaps never overlooked, and that is the so-called "head tetanus." If the infected area, although it be of limited extent, lies on one side of the face, then only the muscles of that side will respond with local tetanus; in such instances even the trismus may be one-sided. In case the infecting wound involves the top or bridge of the nose, the muscles on both sides of the face will be equally affected. The surgeon Brunner has described, in several very excellent papers, not only the cases of head tetanus that he himself has observed but also a large number of other cases that he has collected from the clinical literature on this type of tetanus. This investigator has also made many striking experiments on animals with toxic filtrates or virulent cultures, in which he was able to repeat and demonstrate every feature of head tetanus as presented by his patients.

MODERN IDEAS ON THE METHOD OF DISTRIBUTION OF TETANUS TOXIN

Long before it was known that tetanus is of infectious nature many clinicians had come to believe that it is truly a disease of the central nervous system. As soon as its true cause had been established, physiologists, pharmacologists, neurologists, pathologists, and students of medicine and surgery began an intensive study of the disease by experimental methods. For them, as for many of the earlier clinicians, its more striking symptoms pointed unerringly to an involvement of certain parts of the spinal cord, medulla, and pons. But that well known symptom called local tetanus presented difficulties in the way of its explanation that could not easily be harmonized with the theory that the disease is solely one of central character. So it came about that the problem that mainly occupied the minds of clinicians and experimentalists forty years ago and has continued to do so down to our day may be formulated in the following words: What single theory will explain both local tetanus and the more generalized or reflex tetanus that is finally superimposed upon the former? Can a mode of carriage of toxin to the central nervous system be proved to exist that will serve to explain not only local tetanus but also that of central origin? Naturally, also the following question stood in closest relationship to the problem as here formulated: Is the toxin transported to the reacting cells of the central nervous system by the blood stream, as is the case with alcohol, numerous alkaloids, and many

other substances, or is there an alternative mode of transportation to the central nervous system for such agents as our bacterial toxin? Neglecting for the moment differences of detail, we may say that a theory was finally evolved which is universally accepted in our day. *The proponers of this theory state most positively that the toxin cannot be taken up from the blood stream by reacting cells of the central nervous system, but that it can reach these cells only when it is carried to them by the peripheral nerves.*

EARLIER EXPERIMENTS IN FAVOR OF THE THEORY OF CARRIAGE OF TOXIN IN THE PERIPHERAL NERVES TO THE CELLS OF THE CENTRAL NERVOUS SYSTEM

The theory that drugs can be carried in peripheral nerves directly to cells of the central nervous system was advanced as long ago as 1867 by the physiologists von Bezold and Hirt, who declared that when the alkaloid veratrine is injected into the sciatic nerve of a frog it passes upward in this nerve with a certain rapidity to reach centers of the spinal cord. Thirty years later, J. Ioteyko concluded from her experiments that, when veratrine is applied directly to the sciatic nerve of a frog, it is rapidly transported downward, in or along the nerve, to a muscle that has been left attached to it. Within two minutes after the application of the alkaloid to the nerve the muscle is thrown into fibrillary contractions, and four to six minutes later a muscle tracing characteristic for veratrine is obtained on faradic stimulation of the poisoned nerve. The author concludes as follows: "Cette action de la vératrine ne peut être expliquée que par le transport du poison le long du nerf; la forme caractéristique de la courbe s'obtient non parce que la vératrine a agi sur le tronc nerveux, mais parce qu'elle s'est propagée le long du nerf et est venue intoxiquer le muscle, en sorte que l'excitation indirecte touche un muscle qui contient de la vératrine."

The experiments of von Bezold and Hirt and Ioteyko with veratrine and the conclusions that were drawn from them were submitted by H. Mies to an experimental and critical analysis in an excellent paper on the blood supply of the sciatic nerve of the frog. It was shown by this investigator that even when either the common iliac or the sciatic artery is ligated at the customary points, as in Claude Bernard's operation, the nerve continues to be well supplied with blood by intrapelvic arteries that enter it above the point at which the main artery was tied. Mies therefore very properly challenged the validity of the conclusions at which von Bezold and Ioteyko had arrived, and pointed out that these authors had failed to take into account the ease and rapidity with which the powerful alkaloid used by them can be carried away from the injected nerve for general distribution throughout the body, even after the sciatic artery had been ligated. Mies, how-

ever, made no reference whatever in his paper to the fact that peripheral nerve trunks are richly supplied with lymphatic capillaries and efferent vessels and that these may be even more efficient than the blood capillaries in removing such substances as tetanus toxin from an injected nerve.

INVESTIGATIONS IN SUPPORT OF THE NERVE TRANSPORT THEORY SINCE 1884 AND THE PROPOSED MODIFICATIONS OF IT

It is impossible to say whether the experiments of von Bezold in 1867 on the supposed carriage of veratrine in peripheral nerves influenced investigators on tetanus nearly thirty years later, and encouraged them to believe that tetanus toxin could be carried in peripheral nerve trunks to the central nervous system. But as long ago as 1884, Carle and Rattone injected into the sciatic nerve of each of four rabbits a suspension of purulent material taken from the infiltrated tissues around an infected acne pustule of a patient who had just died of tetanus, and found that the animals developed the most intense symptoms of tetanus after an incubation period of two or three days. They found, in fact, that such injections of infected material into a nerve trunk were as effective in inducing tetanus as when made directly into the rabbit's dorsal muscles. These investigators were also the first to inoculate rabbits [two] with emulsions of sciatic nerve taken from other rabbits that had been made tetanic by injection of the original tetanogenous material from their patient. Here also: "Il risultato fu uguale; in uno però l'opistotono era meno accentuato."

After it had been fully demonstrated, during the years 1884 to 1890, that tetanus in man and animals is a disease of bacterial origin, by the work of Carle and Rattone in 1884, Nicolaier in 1884, Rosenbach in 1887, Kitasato in 1889, Knud Faber in 1890, and by many others in 1889 and 1890 who corroborated the discoveries of these pioneers, Bruschettini made the important observation in 1892 that, when toxic filtrates of the bacillus tetani are injected underneath the skin of animals, the toxin is found to be present in the nerve trunk in the neighborhood of the injected area. This investigator seems to have been the first to suggest that the toxin of tetanus can be transported from the point of injection in both a centripetal and a peripheral direction in nerve trunks. Meyer, however, calls particular attention to the fact that Bruschettini failed to draw such deductions as could

have been drawn from his experiments in respect to the possibility of their furnishing an adequate explanation for local tetanus, as was done later by him and Ransom. Bruschettini, however, may truly be said to have found the path that has been so well trodden by his successors.

In 1903 Morax and Marie injected the enormous quantity of 3 grams of a powerful tetanus toxin (of which 0.0000005 gram killed mice weighing 15 grams) into the gastrocnemius muscle of the left hind leg of a pony weighing 334 kilograms. The dose injected into this pony, according to our calculations, represented on the most generous estimate not less than 1000 fatal doses for the animal. The pony died of tetanus a little less than three days after inoculation with the toxin. After its death the authors estimated the amount of toxin present in the blood, in a great many nerves of the body, in parts of the central nervous system, in the cerebrospinal fluid, aqueous humor, and elsewhere. The toxicity of these various tissues was determined by injecting extracts of macerated portions of them into mice. The authors found the poison to be present in the blood, in peripheral nerves and parts of the central nervous system, and in the left crural muscle. The sciatic nerve of the left or inoculated leg contained weight for weight more than the corresponding nerve of the opposite side. We cannot consider here in greater detail the analytical reports of Morax and Marie either in reference to their pony or to similar experiments that were made by them on a monkey. Suffice it to say that these authors found that they could demonstrate the presence of the toxin in all classes of nerves, and they therefore concluded "that the three types of peripheral neurons, the motor, the sensory and the sympathetic, are equally able to absorb the tetanus toxin." They finally assumed, however, that the toxin is not carried to the central nervous system either by sensory or autonomic nerves but only by the fibres of motor nerves. It may be remarked in passing that Morax and Marie point out that in a given weight of the sciatic nerve, as for example, when 0.05 gram of the nerve killed mice of the usual weight in two or three days, the amount of toxin contained in this bit of nerve would be exactly what calculation showed should be present in it if the poison were equally distributed throughout the blood and every organ of the body. It is plain, therefore, that even when such enormous quantities of the toxin are injected as in the

experiments of the French authors, no actual concentration of the poison occurs in peripheral nerves.

Meyer and Ransom entered the field at about the same time as Marie and Morax, though Meyer's first paper appeared a year before that of the French authors. Important contributions were made by Loewi and Meyer and Fröhlich and Meyer, and by W. M. Fletcher, who carried on his work in Meyer's laboratory. The investigations of Meyer and his collaborators and the conclusions that were drawn from them have played a leading rôle among the theories of the nature of tetanus that have been proposed during the past thirty years. In all of the papers from Meyer's laboratory the assumption that the tetanus toxin can reach the reacting centers of the central nervous system only by being carried to them in the axis cylinders of motor nerves has been strongly defended by means of well designed and skillfully performed experiments. To many who have not themselves worked in this field this theory of such an unusual and exceptional mode of carriage of a toxin has appeared to be entirely valid and completely irrefutable.

In the hands of Meyer and many later experimenters who gave it their hearty support the theory seemed to give a good explanation of the causation of local tetanus. It has been stated above that this symptom, which may be of long duration, is readily induced in even a single muscle of an extremity when a very minute amount of the toxin is injected into it. The resulting contracture of the muscle is readily explained by the Meyer-Marie theory. The toxin is taken up by the many motor nerve fibres that are dispersed throughout the muscle and is then carried in them directly to motor cells of the anterior horn of the cord. These nerve cells, once the toxin has reached them, now pass into a hyperexcitable state; they become more responsive to sensory stimuli and constantly send out excitatory impulses to the muscles under their control. The appearance of local tetanus is thus fully accounted for; it can occur only *after* the intramuscularly injected toxin (as in our assumed case) has been carried to the central nervous system along this intraneural pathway. This mode of carriage was now generalized to apply to every motor nerve fibre throughout the body. The lymphatics carry the toxin from an infected area into the blood stream, which in turn distributes it to all of the voluntary

striated muscles of the body, and thus serves merely as an agency for supplying toxin to the absorbing and transporting motor nerve fibres of these muscles. The central nervous system cannot take up toxin directly from the blood, but only from the motor axons of peripheral nerve trunks. In other words, the toxin is incapable of passing the "blood-brain barrier." The theory appeared to receive verification from the results of intraneural injections of toxin and of nerve transections both before and after injection of toxin into an extremity. Since the results of such procedures serve equally well to support the other variants of the neural transport theory, we shall consider their evidential value in connection with our discussion of these variants.

Meyer also found strong support for his special theory by reasoning in the following manner. There is but one known continuous path that connects a muscle fibre or an assembled packet of muscle fibres—a so-called motor unit—and the corresponding motor cells of the central nervous system. This pathway is represented by the axons of the motor nerve cells. We have seen that a single muscle can be thrown into local tetanus by injection into it of a minute amount of toxin. Passage of the toxin in these axon pathways meets the requirement of the sharp "one to one correspondence" between a contracture of a motor unit of muscle fibres and the exciting impulses that emanate from its controlling central nerve cells. This very logical way of thinking (the premises being thought sound) induced Meyer to discard an alternative mode of transportation of the toxin, namely, by way of the lymphatics of nerve trunks. He reasoned that if the toxin were conveyed centrally by the lymphatics of a nerve it could not possibly be so precisely apportioned among the limited number of motor nerve cells of the spinal cord that induce local tetanus in a limited number of muscles and only in those that are innervated by the poisoned anterior horn cells.[3]

[3] Goldscheider (Ztschr. f. klin. Med., 1894, xxvi, 175–189) had already stated in 1894 that one is at a loss to explain how the exact localization of the toxin, in the very limited portion of the central nervous system responsible for local tetanus, could be explained by carriage in the lymphatics of peripheral nerves to the subdural and subarachnoid spaces of the central nervous system, and for this reason he refused to accept the nerve transmission theory of toxin. He believed that the

The student of tetanus soon finds that there are wide differences of opinon in regard to almost every feature of the disease. This holds true particularly for the nerve transport theory, as has already been intimated above. There are three variants of this theory: (1) carriage in the nerve fibres themselves—the Meyer-Marie variant which we have been discussing, (2) transport of the toxin from the periphery to the central nervous system and cerebrospinal fluid by endo- and perineural lymphatic vessels of mixed nerves, and (3) centripetal transport in the tissue spaces or *Lymphräume* of nerve trunks.

Let us examine each of these assumed variants of the nerve transport theory more carefully.

1. Further Remarks on the Theory of Carriage in Motor Nerve Fibres

We have already made some observations of a critical nature concerning this variant of the general theory but more must be added. Meyer and his associates state very definitely that this mode of carriage can proceed only very slowly and that the toxin is transported centripetally as well as centrifugally in axis cylinders by a process that is termed "protoplasmic streaming." It may be pointed out that there is no experimental evidence whatever that there exists anything in the nature of a slow "protoplasmic streaming" or current that can carry the molecules of the toxin from point to point in axis cylinders, as is the case when it is injected into a vein or a lymphatic vessel with its moving current. Secondly, when the toxin is found to be present in an excised piece of nerve, such as the sciatic, in the neighborhood of the point of the injection, there is no trustworthy evidence that an appreciable amount of it *is present in the axis cylinders themselves* and that the blood capillaries and the endo- and perineural lymphatic vessels of the nerve do not contain practically all of it. It

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primary local tetanus and the later more generalized tetanic convulsions are both to be explained by a central action of the toxin, and he also assumed that the toxin could so alter the state of excitability of axon terminals that their corresponding neurons in the cord would in some unexplained manner be themselves rendered more excitable by this action of the toxin on their peripheral terminations in the muscles.

is well known that the lymphatic capillaries readily absorb the toxin from any injected area and carry it into the blood stream, and there is no sound reason for believing that those of a nerve trunk do not also absorb it if given the opportunity. Too much has been made by the proponents of this variant of the nerve transport theory (as well as by those who champion its other variants) of the well known fact that the part of a nerve nearest the point of injection can be shown to contain some of the toxin. Sawamura, an ardent supporter of the neural transport theory, admits that no part of the sciatic nerve can be shown to have taken up the toxin unless at least two fatal doses of it have been injected subcutaneously into the leg, and that it is always found only in that part of the sciatic nerve that lies nearest to the point of injection of the toxin. Both Permin, also an ardent supporter of the nerve fibre transport theory, and Pochhammer state that after the injection of toxin into the nerve of a leg its presence cannot be detected in the upper part of the sciatic nerve unless large quantities of it have been injected. Permin succeeded in finding it only in the upper part of the sciatic of a large guinea-pig (experiment of July 6, 1911, page 22 of his paper) after injecting into the calf of its left leg so large a quantity of toxin as to kill the animal in twenty-four hours! He is much puzzled to explain the absence of the toxin in the upper part of the nerve after the injection of several fatal doses into the leg, and suggests that this is due to the rapid upward carriage of the toxin. He forgets that in accordance with his own observations intraneurally injected toxin rapidly escapes from the nerve into the surrounding tissues and muscles and that after a time the nerve itself contains no more toxin than the tissues immediately surrounding it. The fact that the more centrally-lying parts of the sciatic are found to contain no toxin unless heroic doses have been injected into a leg is now readily explainable. The many efferent lymphatic vessels of the upper portions of a nerve trunk constantly remove whatever part of the toxin has been taken up by the lower portion of the nerve. This fact cannot be reconciled with the theory of the carriage of toxin to the spinal cord in the neural lymphatics.

Pochhammer, who has propounded the singular theory that local tetanus is brought about by an action of the toxin in destroying the myelin sheath of nerves, both motor and sensory, and that these

bared nerves now permit the direct passage of a sensory impulse *from a bare sensory nerve* to a similarly bare neighboring motor nerve, thus producing contracture of muscles by a process of short-circuiting of impulses, makes the following claim on the basis of his experiments. He states positively that local tetanus appears in the muscles of the hind leg of a rabbit that has received a subcutaneous injection of the toxin *before* the toxin can be detected in the sciatic nerve itself. It is quite impossible to bring this and other observations mentioned above into line with the theory of a steady upward movement of the toxin in nerve fibres.

It has been stated that Meyer believes that the carriage of toxin in nerve fibres proceeds very slowly. In his opinion and in that of his later defenders this very slowness of transport affords a good explanation for the well known period of incubation that always intervenes between the injection of toxin and the appearance of the first symptoms of tetanus thereafter. Singularly enough, however, other competent investigators disagree sharply with Meyer. The surgeon Permin describes in detail certain experiments on rabbits that have led him to believe that the transport of toxin in nerve fibres proceeds not slowly but with great rapidity ("ausserordentlich rasch"). According to him it requires only four to six hours in the rabbit for the toxin to pass from the distal part of its sciatic nerve to its spinal cord. Sawamura, who has worked very intensively at tetanus, came to much the same conclusion as Permin but some years earlier. He concluded from experiments much like those performed by Permin that the conduction [of the toxin] in peripheral nerves proceeds "pretty rapidly" in them, according to his observations. ("Die Fortleitung in peripherischen Nerven geht nach meinen Untersuchungen ziemlich schnell.")

Quite aside from this difference of opinion among investigators as to the rate of carriage of the toxin in nerve fibres, it is generally believed even in our day that the period of incubation is much longer in the larger animals, such as the horse and man, in which it may vary from three days or even less to twenty or more days, than is the case in very small animals. In the mouse and guinea-pig, after experimental inoculation, the period may be as short as from eight to twelve hours; in the rabbit it is set down as varying from eighteen to thirty-six

hours. Treatises on tetanus give tables of varying periods of incubation for animals from the size of the mouse to that of the horse, such as the one drawn up by Courmont and Doyon. In these tables the period of incubation is always given as one of hours in the small animals, and then as increasing progressively until it reaches three to five days or more in the larger animals. Data of this kind were thought by Meyer and others to support their theory, as it is quite evident that the toxin would require much more time to ascend the long sciatic nerve of a horse, for instance, than the much shorter sciatic of a guinea-pig. But the belief that the period of incubation is always of short duration in the very small animals, as compared with the large ones, does not rest on a firm foundation. It has been found in our laboratory, for example, that a properly selected subcutaneous dose of toxin will give for the guinea-pig the same incubation period of three or four days as a similarly selected dose will give for so large an animal as a sheep weighing three hundred times as much as the guinea-pig. Many factors must be taken into consideration in making positive statements concerning the length of the incubation period in the case of any animal species. Similar factors come into play in causing the extremely variable periods of incubation as observed in the natural disease in man and in the equidae. The amount of toxin produced at the site of infection, or of that inoculated into an experimental animal, its virulence, the locus of infection or inoculation, whether this be in, close to, or at a distance from muscles, the amount of daily labor that is done by a human being or the amount of running about that is done by an animal after infection or inoculation and many other factors, all have a marked influence on the length of the period of incubation.

One of the most important determining factors is undoubtedly the amount of toxin that is injected at any one point of the body. This was well shown by Morax and Marie in their experiments on a pony, referred to above. It will be recalled that a very large dose of a powerful toxin, amounting to 1000 fatal doses or more, was injected into the gastrocnemius muscle of a pony's left leg. On the morning of the following day local tetanus had already made its appearance in the injected leg, and lockjaw and general tetanic convulsions followed rapidly. We have here, then, an example of the great shortening of the incubation period after *intramuscular* injection of a very large dose of the toxin to twenty-four hours or less. But it is after the *intravenous* injection of large doses that the shortest

periods of incubation are found. Thus Marie, who pointed out as long ago as 1897 that the period of incubation "is essentially variable and depends in particular on the quantity and activity of the tetanic poison," found that a rabbit that had received intravenously half a milligram of a very active and standardized toxin died of generalized tetanus on the fourth day, while a second rabbit on receiving 100 mgm. of the same toxin, also intravenously, died of tetanus in twelve hours. Our own experiments with guinea-pigs show that these animals will succumb to generalized tetanus within eight to ten hours following direct injections into the heart of 8,000 to 20,000 lethal doses. After the injection of such large amounts the animals are apparently quite unharmed and show no noticeable manifestations of tetanus until seven hours have elapsed. Then there appear quite suddenly the extreme symptoms characteristic of the disease, and these persist until death occurs two or three hours later. Two control animals injected in the same way, one with an equal quantity of the uninoculated medium and the other with a toxin that had been boiled to destroy its potency, showed no ill effects during an observation period of two weeks.

It was stated by Marie that the minimal intravenously injected lethal dose of the toxin for a rabbit is eight to nine times larger than that required to kill one of equal weight into which the toxin is injected under the skin; while Knorr found that the intravenous minimal lethal dose is twice that of the subcutaneous. Quadu asserts that in guinea-pigs the intravenous lethal dose is six times larger than the subcutaneous. Fildes found that the relation for the rabbit is as five to one when the intravenous mode of injection is compared with that into the muscles. This supposed striking difference between the lethality of intravenous and other modes of injecting moderate amounts of the toxin is surprising and quite out of line with the results of similar injections of many poisons, such as alkaloids, glucosides and other pharmacological agents.

A year ago van den Hoven van Genderen for the first time in the history of tetanus gave serious attention to this matter and by the use of a sufficiently large number of animals obtained data that carry conviction to the reader's mind. He finds that in the case of rabbits and guinea-pigs, quite contrary to the belief of earlier investigators, no material difference can be established in the time of death following intravenous, intramuscular, or subcutaneous injections of from one to three or four lethal doses of toxin, expressed in terms of guinea-pig minimal lethal doses. We ourselves had already arrived at the same conclusion in experiments on animals other than those studied by van den Hoven van Genderen though our evidence was based on a smaller number of cases. Our experiments with sheep and with dogs have shown that when one injects from one to four or five lethal doses, while there may be slight differences in the time of the first appearance of symptoms, the time of death is practically independent of the mode of injection. We have purposely omitted here all reference to intracerebral or intraspinal injections of the toxin. Their significance can be discussed more profitably in a later paper in which local tetanus with and without reflex or central nervous involvement will be described.

The data given above seem to prove that the shortest periods of incubation in all animals thus far tested attend the intravenous injection of very large doses of the toxin, such, however, as would probably never come into play in the natural disease. An interesting feature of the physiological action of the toxin is seen in the fact that even when very large doses of the poison are injected intravenously no alteration of blood pressure, respiration or the general condition of the animal is discernible until many hours have elapsed. Courmont and Doyon tested this matter on a dog by injecting as much as 258 cc. of tetanus toxin (143 lethal doses) into the jugular vein at a rate of 1 cc. every 18 seconds, and found no changes in blood pressure, cardiac rhythm or respiration, during the three hours the animal was on the operating table.[4] After detaching the manometric apparatus the animal was found to be quite "normal" and two hours later (9.00 P.M.), when the last observations of the day were made, it presented no noticeable symptoms of poisoning. On the following morning a slight incoördination of gait had appeared. At 1.15 P.M. the forelegs and the neck were stiff; a few hours later the animal died in general convulsions. Here again in this experiment of Courmont and Doyon we find that intravenous injection of an excessive amount of toxin is followed by an incubation period of hours, and that death inevitably occurs shortly after the first appearance of rigidity in the extremities.

We believe, then, that as in the natural disease in man, so also in experimental work on animals, one cannot assign a definite lower and a definite upper limit to the period of incubation, and that variations in this period are readily accounted for by taking into consideration the factors just enumerated. Nevertheless, one fact stands out prominently both in the natural disease and in experimental work with animals, namely, that under ordinary conditions in which only from one to two fatal doses of toxin come into play a number of days supervene between natural infection or subcutaneous inoculation and

[4] Meyer ("Tetanus Studien," in Jaffe's Festschrift, p. 306) also measured the blood pressure of a number of dogs in advanced stages of tetanus and found that it differs practically not at all from the normal blood pressure of dogs. In tetanus he found that it varied from 130 to 140 mm. Hg. During the convulsions it rose to about 140 mm., sank thereafter for a short time to about 120 mm. and then rapidly regained its original level of 130 to 140 mm.

the first symptoms of the disease. In other words, the poison with which we are here concerned manifests its action *slowly* in all species of animals that are susceptible to its action except under quite exceptional circumstances, as when truly enormous doses of it are injected intravenously. We have pointed out that one can make the period of incubation in any species of animal either short or long, and that tables that attempt to set definite limits for the period have little significance from a scientific point of view and none whatever in the way of lending support to the nerve fibre transport theory. We have marshalled much evidence against the validity of this theory, but many more facts that cannot be brought into harmony with it or the two other variants of the neural transport theory will be presented incidentally in later papers dealing at greater length with local tetanus and other features of the disease.

2. The Toxin Is Supposed to Be Carried to the Central Nervous System by Way of the Endoneural and Perineural Lymphatic Vessels

This second variant of the nerve transport theory had its early proponents in Gumprecht, Stintzing, Cyrus Field, and others, and was strongly supported by the investigations of the pathologist Aschoff and his collaborators Reinhold and Robertson that were made during the world war. Teale and Embleton, whose investigations also fell in that period, likewise accept this second or lymphatic mode of transport in addition to carriage by nerve fibres. Horster and Whitman have made many experiments as recently as 1931 to show the possibility of this mode of carriage of the toxin, and their work has been cited with approval in this very year by Friedemann and Elkeles as supplying corroborative evidence "for the conception that the toxin reaches the spinal cord and the brain exclusively by way of the peripheral nerves." It is evident that this postulated mode of conveyance of the toxin to the submeningeal spaces in the closed channels of the lymphatics cannot be regarded as one that proceeds very slowly, as was assumed to be the case by Meyer and his school for their theory. Once the toxin has been taken up by the lymphatics of the peripheral portions of a nerve trunk it must proceed centripetally at the rate of flow prevailing in these vessels. This second variant of the neural transport theory, in contradistinction to the first, offers no explanation

for the long period of incubation[5] that so often precedes the appearance of local tetanus in an extremity that has been injected with the toxin. Were this theory correct, one could certainly expect a quicker central response to the action of the toxin with a more rapid appearance of convulsive seizures. Investigators that defend this second, like the defenders of the first variant of the theory, rely to a very great extent on injections of toxin into nerve trunks for their evidence, and they appear to be firmly convinced that all neural lymphatics have a free entrance into the cerebrospinal fluid and the substance of the cord. Their ideas are based in large measure on the monumental investigations made in the early 70's of the last century by Key and Retzius. As is well known, these investigators injected gelatin solutions containing Berlin blue into the subarachnoid cavity of human cadavers and found that the blue salt found its way along peripheral spinal nerves to a considerable distance from the spinal cord. They concluded that definite pathways exist along which the cerebrospinal fluid can easily pass into the perineural spaces and even into the very substance of peripheral nerves. It may not be out of place to cite a passage in which they describe the distribution in peripheral nerves of the material that had been injected into the subarachnoid cavity. "Ins innere der Nervenbündel setzt sich ferner oft die Flüssigkeit von den perineuralen Spaltenräumen mehr oder weniger zwischen den an gewissen Stellen sich einbiegenden endoneuralen Lamellen in die endoneuralen Spaltenräume fort. Sie breitet sich dabei in grösserer oder geringerer Ausdehnung in den inneren Theilen des Bündels aus, indem sie erstens hauptsächlich zwischen den sich immer weiter vertheilenden Lamellen verläuft und die Nervenfasergruppen in dieser Weise umspült. Die injicirte Flüssigkeit bleibt aber nicht in den endoneuralen Spaltenräumen, sondern breitet sich auch hie und da von denselben in die Nervenfasergruppen selbst aus. Ihr Verhalten zu letzteren ist etwas eigenthümlich. Sie bleibt nicht in den Spalten ausserhalb der Fibrillenscheiden, noch dringt sie nur in diese hinein, sondern sie füllt den ganzen Raum ausserhalb und innerhalb der Fibrillenscheiden, bis zu den Schwannschen Scheiden."

The anatomists of our day have become very skeptical of the con-

[5] We shall offer our own theory in explanation of the period of incubation in a later paper.

clusions that were drawn by Key and Retzius from their experiments. They believe rather that the high injection pressures employed by them on cadavers (about 60 mm. Hg.) account for the escape of their Berlin blue solution in the subarachnoid cavity far out along the spinal nerves. Robert Elman, working in the laboratory of Professor Lewis H. Weed[6] of the Johns Hopkins University, has made careful histological studies of the region of the spinal cord where the spinal nerve roots emerge, after subarachnoid injections of isotonic solutions of a non-toxic substance, and has not been able to demonstrate "the existence of a fluid pathway physiologically connecting the subarachnoid cavity with the so-called perineural space."

Iwanow and Romodanowsky corroborated Elman's findings a few years later as a result of their own studies "on the anatomical connection of the cerebral and spinal submeningeal spaces with the lymphatic system," without, however, being acquainted with Elman's work. After injecting especially prepared suspensions of India ink and certain non-diffusible colloids into the subarachnoid spaces of both living and dead animals, these investigators found that the injected substances were not carried away from the subarachnoid cavity by the spinal nerve roots. Microscopic examination of the roots showed that they had taken up only a certain amount of the India ink but this was never carried farther in them than the intervertebral ganglion. So far these authors are in agreement with Elman, but they now go on to say that their observations support the well known belief of Speransky's school that "fluids travel in nerves (*Nervenscheiden*) toward the brain and not in the reverse direction." Elman gives no hint whatever in his paper that he subscribes to this mode of transfer of fluids in peripheral nerves.

We cannot here do more than allude in passing to the main objective of Iwanow's own researches and those made in collaboration with Romodanowsky. These authors believe that they have realized their objective and have discovered that a not inconsiderable part of the cerebrospinal fluid escapes from the subarachnoid space in lymphatic

[6] Professor Weed has made some sound critical remarks on the observations of Key and Retzius in relation to the drainage of the cerebrospinal fluid, in his "Studies on Cerebrospinal Fluid," published in the Journal of Medical Research, Boston, 1914, n.s. xxvi, no. 1, p. 45.

vessels of a special type that have their origin in the dura mater and is carried in them to many of the abdominal and thoracic lymph glands and to the intestinal mucosa and Peyer's patches. Since it is assumed by these writers that this flow of cerebrospinal fluid in special lymphatics is centripetal and unidirectional only, we need not concern ourselves further with it and may leave it for anatomists to decide whether this mode of drainage of cerebrospinal fluid actually occurs under normal circumstances. Acquiring our information from others we had assumed that a lymphatic system has its origin in the lymph spaces of the cerebral layer of the dura mater; that it drains into the superior deeply-lying cervical lymph nodes; that "*this system is closed, however, on the cerebral surface of the dural membrane and has no communication with the pia-arachnoid spaces.*"[7] (Turner and Reynolds, p. 12.)

Implications of the Theory

The theory of a free flow to the central nervous system in the neural lymphatics now under consideration implies, in the first place, that all the lymph of peripheral nerves mingles ultimately with the cerebrospinal fluid, and secondly, that any substance whatever, be it a useful drug or a toxin, that finds its way into peripheral nerves and is devoid of destructive action on them must finally reach the central nervous system. In recent years Speransky and his pupils Wischnewsky and Ponomarew have gone so far as to make the following statements in regard to these matters. Thus Speransky writes, "Il s'ensuit donc que les liquides du tronc nerveux montent, quoique lentement, mais sans interruption, et avec une vitesse variable, dans la direction du cerveau et s'insinuent partiellement dans le segment correspondant. Ils emportent dans leur courant des substances de composition chimique très variée, qui y ont pénétré d'une facon quelconque." And his pupil Wischnewsky states that[8] "the lymph of a nerve trunk flows in a steady slow stream in the direction toward the brain and carries with it all substances that have entered it, provided these substances are indifferent in their action on local tissues and do not destroy their structure. Not only tetanus and

[7] Italics ours.

[8] Translated from the German.

diphtheria toxin but also many other substances which, in this or that manner, have entered a nerve trunk are carried forward passively in the nerve trunk. The forward movement (*Fortbewegung*) of the lymph in a nerve trunk, like the movement of other fluids in the body, depends upon the difference of pressure at the beginning and end of the way. By raising or lowering this pressure at the beginning or end of the route (*Bahn*) the rapidity of transfer of the fluid in a nerve can be voluntarily altered." In all of these statements the end point of this flowing current in peripheral nerves is always found in the central nervous system. Many experiments have been made by Speransky's pupils, Ponomarew and Wischnewsky, in support of these surprising statements. They have injected dye stuffs and tetanus toxin into nerve trunks and have attempted to note changes in their rate of passage after altering the pressure of the cerebrospinal fluid either by withdrawing various amounts of it or by an ingenious operative procedure, in which a cuff of muscle tissue is placed around the spinal cord itself in such a manner that the upper part of the spinal subarachnoid cavity is completely cut off from its lower division. All of these experiments, it will be noted, and the deductions that are drawn from them are based on the underlying assumption of a free passage of fluid from nerve trunks to the subarachnoid spaces. We shall not consider at this point in further detail the experimental findings of these authors, since their assumption will now be shown to be baseless.

It is surprising that none of the proponents of the theory of carriage of toxins and other drugs by way of the neural lymphatics have ever asked themselves the simple question whether their assumption of a physiologically occurring centripetal flow of lymph in nerve trunks has any foundation in fact. They seem never to have inquired whether the anatomists of our day have studied this matter and whether they have discovered at what points the lymph of nerve trunks leaves them and where it goes. In 1930 Aldo Defrise, in numerous neural injection experiments on newborn babies and on dogs, studied the mode of origin of the lymphatic capillary networks of peripheral nerves, and came to the conclusion that this network is composed of a closed system of lymphatic capillaries which are not open to the interlamellar spaces of the perineurium. He studied the epineural efferent lymphatic "collectors" of the median, crural, sciatic,

peroneal (communis), and posterior tibial nerves, tracing these lymphatic vessels to the lymphoglandular groups into which they empty. We cannot here enter into further details of this anatomist's work and the precautions that he has taken in regard to the materials used in his injections, pressures, and other points of technique. Jossifow and his associates Shdanow and Kurdümow have also published very extensive researches in this field since 1930. Shdanow has traced the course of the lymphatics of the nerve trunks of both the fore and hind limbs of man. The efferent lymphatics of the nerves of the hind legs, for example, take their course along the arteries of the nerve and throw their contents into more deeply-lying "collectors," but in all cases the lymph finally passes through regional lymph nodes onward into the thoracic duct. Nowhere in the writings of the anatomists here cited is there even a suggestion that any neural lymphatic vessels enter the spinal canal at any intervertebral junction. The lymphatics, for example, at the upper part of the sciatic reach deeply-lying inguinal glands and those of its lumbo-sacral trunk finally empty into the hypogastric lymph glands. In a word, we derive no evidence from these recent studies of anatomists that the lymphatic vessels of nerve trunks pour their contents into the submeningeal spaces of the central nervous system, and it would seem to be quite impossible longer to maintain that tetanus toxin or any other substance that may have been taken up by the neural lymphatics can be carried in them to the central nervous system.

We may remark in passing that we are leaving out of consideration all reference to the anatomical conditions that exist in the olfactory and optic nerves, as neither of these can properly be classed with the peripheral nerves.

In concluding this section of our paper a few remarks will be in order on the work of Horster and Whitman, alluded to above. These investigators, like their very numerous predecessors (none of whose experiments can be described here), have studied the effects of injecting the sciatic nerves of rabbits with trypanblue and with tetanus toxin and have found that these substances can be driven very rapidly into the cerebrospinal fluid. Thus, in one of their many experiments with trypanblue, in which 0.05 cc. of a strong solution of this dye was injected into the right sciatic nerve of a rabbit at a point 4 cm.

below the spinal cord, it was found that the spinal cord was stained blue for a distance of 2 cm. above the cauda equina. In this case the animal was killed immediately after the completion of the injection and the spinal cord was at once examined. In another experiment (rabbit 15) 0.08 cc. of the trypanblue solution was injected into the right tibial nerve at a distance of 2 cm. above its entrance into the muscle, and in so short a period as five minutes after the injection clonic seizures appeared in both hind limbs. Similar experiments were made with injections of tetanus toxin. One sciatic nerve in each of a series of twelve sizable rabbits was exposed under morphine-ether anesthesia and a concentrated solution of tetanus toxin was injected into the nerve trunk. The volume injected varied from 0.04 to 0.1 cc. These amounts of toxin represented from 4,800 to 12,000 lethal mouse doses. At various periods following the intraneural injections cerebrospinal fluid was removed by occipital puncture and was tested for its content of toxin by subcutaneous injection into white mice. In five out of the twelve experiments the authors found that the spinal liquor was toxic for mice in quantities varying from 0.25 to 0.65 cc. In these successful experiments, as in experiments with trypanblue, the toxin appeared *very soon* in the cisternal liquor. When the cisternal liquor was found to contain no toxin within two to three hours after the intraneural injection, it was never found to be toxic when tested at any time in the following 24 to 48 hours. In a word, when Horster and Whitman did not succeed in *driving* the toxin into the lumbar cerebrospinal fluid at once by syringe pressure, the cisternal fluid never contained any of it later, even when the authors waited almost three days for signs of its appearance. No more conclusive evidence could be offered than that given by Horster and Whitman themselves that the high injection pressure of a syringe can drive dye stuffs and toxin very rapidly into the submeningeal spaces of the lumbar spinal cord. Furthermore, an examination of their unsuccessful experiments, that is to say, those in which they failed to drive the toxin at once into the submeningeal cavity, leads us to a very important conclusion. In all of these unsuccessful experiments the solution was propelled only a certain distance up and down the nerve and not far enough centrally to reach the submeningeal spaces, *and yet in all of the experiments of this nature, although the toxin was dis-*

tributed throughout a certain length of the nerve trunk, it ceased to travel further along the nerve as soon as the pressure of the injection had fallen to a low level. In a word, even when there is a good deal of toxin or dye stuff present in a nerve trunk *it cannot be carried into the central nervous system by forces naturally inherent in the nerve or by its lymphatics.* The authors themselves remark that under these circumstances the toxin "could not reach the submeningeal spaces by a secondary centripetal transport from the intraneural depot." . . . "dass grössere Farbstoff- oder Toxinmengen zwar durch die intraneurale Injektion als solche in den Liquor bzw. in die submeningealen Räume geschafft werden können, nicht aber durch sekundären zentripetalen Transport von einem intraneuralen Depot aus."

3. On the Carriage of the Toxin in the Tissue Spaces of Nerve Trunks

Peripheral nerve trunks, like other tissues and organs of the body that are richly supplied with blood and lymphatic vessels, contain tissue fluid that fills the so-called tissue spaces. Anatomists hold that no true lymphatic capillaries are found in the bundles of nerve fibres that compose a peripheral nerve[9] but tissue spaces, or "*Lymphräume*," exist around every nerve fibre and between the thin layers of the perineural sheath surrounding bundles of such fibres. These minute spaces with their numerous interposed barriers are supposed to continue on in the nerves through intervertebral structures and foramina right into the subarachnoidal and subdural spaces of the spinal cord and brain. They do not join up in any way with lymph vessels that may be present in the epineural tissues. The amount of tissue fluid that is supposed to be present in these minute spaces must be very small indeed in the normal and uninflamed nerve, since peripheral nerve trunks are classed with the less watery structures of the body. There is not the slightest evidence that the metabolites present in the tissue spaces or toxins that have found their way into them can be transported from one point to a more distant one by any but the slow process of diffusion. However, in recent years Speransky and his co-workers Wischnewsky and Ponomarew have asserted, indeed, if we read their papers correctly, that tetanus toxin can be carried to the central nervous system in these neural tissue spaces. It has already

[9] Shdanow: Anat. Anzeiger, 1931, 71, 231–245.

been stated that these investigators accept the theory of carriage of toxin by the lymphatic vessels of nerve trunks, but to this theory they have added the mode of carriage that we are now considering. To quote a paragraph from a summary given by Wischnewsky in one of his papers, "The various chemical agents introduced into the peripheral part of a nerve spread out in the nerve path (Nervenbahn) in a centripetal direction; these substances can not only reach the central part of a nerve but obviously also the spinal cord itself. *The paths by which they spread are the perineural lymph or tissue spaces*[10] (Lymphräume)." The term Lymphräume is invariably used by anatomists writing in the German language as the equivalent of the English term *tissue spaces*. We have then no other recourse than to believe that the Speransky school assumes, in part at least, centripetal carriage of toxins in these minute spaces.

We have seen that the second variant of the nerve transport theory is entirely without foundation, now that it is known that the lymphatic vessels carry the lymph of nerve trunks into lymph glands and then on to the thoracic duct, and we believe that the theory that the toxin is conveyed to the central nervous system by way of the tissue spaces of nerve trunks is equally untenable. The tissue pressure at peripheral points is not of sufficient magnitude to move solutions through such narrow spaces, frequently interrupted as they are by barriers. These minute spaces are energized by molecular forces only (surface energy and diffusion) and it remains to be proved that such forces can compass the astonishing feat of transporting a poison for long distances, as in the sciatic nerve of a horse, and through intervening cell barriers to the central nervous system, in any period of time that could ever come into consideration either under experimental conditions or in the naturally occurring disease, tetanus. Diffusion can be effective only in a short space of time when it operates in structures of minute dimensions, as in pulmonary alveolar capillaries and other absorbing cell layers whose thickness is only about a thousandth of a millimeter.

We have already emphasized the well known fact that there is only one method known to physiological science by which a substance can be *rapidly* transported from one point to another of an organ, and that

[10] Italics ours.

is by way of the blood or lymphatic circulation. It has also been pointed out that tetanus toxin or a dye stuff, such as trypanblue, can be introduced into a peripheral nerve trunk under low pressure and that, although under these conditions the tissue space mechanism of the nerve is given every opportunity to operate, it is nevertheless entirely unable to carry anything into the subarachnoid spaces of the central nervous system. What part of the injected substances is present in these tissue spaces of the nerve is soon carried away from them by blood and lymphatic capillaries.

We need not spend more time in discussing this question of the assumed, more or less rapid movement of solutions in the tissue spaces of long peripheral nerves.

DISPROOF OF AN EARLIER ATTEMPT TO SHOW THAT WATER SOLUBLE SUBSTANCES CAN BE DISTRIBUTED THROUGHOUT THE BODY BY THE "TISSUE SPACE MECHANISM"

It is a curious fact that this theory appears to intrigue investigators from time to time, and it may be well to comment briefly on the work of S. J. Meltzer, who thought he had proved the existence of a perfect mechanism for the distribution of substances throughout the tissue spaces of the entire body. In 1911 this investigator, in commenting on his numerous experiments with alkaloids and acid fuchsin on frogs said "My experiments demonstrate that in the absence of central circulation substances may be distributed through the body by a mechanism which in some instances may act even more promptly then the cardiac mechanism. In contradistinction to the central apparatus we may designate the distributory agent in question as the peripheral mechanism. The path of distribution employed in this mechanism can be nothing else than the tissue spaces." Meltzer's work was done on cardiectomized frogs, which were shown to react, in the entire absence of the circulation, with great rapidity toward very small quantities of acid fuchsin, strychnine and morphine. In fact it was found that the effects were much greater and appeared more rapidly than when the drugs were administered to normal frogs.

As the senior author of this paper and his collaborators had been working on the action of convulsive dye stuffs on frogs before Meltzer

published his theory and were engaged in their investigations at the time of its appearance, they could not do otherwise than put his theory to the test of experiment. It was found that Meltzer had failed to take into account the rôle that was played in his experiments on cardiectomized frogs by their still actively beating lymph hearts, in the carriage of his drugs from the injected area to the central nervous system. When the four lymph hearts of a cardiectomized frog are completely destroyed or excised no effects whatever follow the injection of any centrally acting dye stuffs or alkaloids into a subcutaneous lymph sac. It was furthermore shown that Meltzer's supposed peripheral mechanism was utterly incapable of distributing any substance whatever *throughout the entire body of a cardiectomized frog, when all of its lymph hearts were intact.* In fact, the intact lymph hearts of Meltzer's cardiectomized frogs were able to transfer substances that had been injected at any point beneath the skin *to one organ only, namely, the spinal cord and brain.* It was found that, when the heart is tied off, the beating lymph hearts can take over only a part of the distributory rôle formerly played by the cardiac mechanism; they can carry drugs with an almost equal rapidity to the central nervous system, but only into this division of the frog's body. The explanation was found in the fact that although the lymph hearts can no longer push their lymph into the heart, since this organ has been tied off, they can still send it directly into the system of veins that drain the central nervous system. In this manner, acid fuchsin, strychnine and the like, that have been taken up by lymph hearts, are carried directly in these veins to the meninges of the brain and spinal cord, and this *reverse current* is made possible by the fact that these veins are entirely valveless.

The reader will find many more data in the experiments that were carried out by the writer and his associates in the years 1910 to 1914 that will convince him of the utter ineffectiveness of "the peripheral mechanism" as a distributing agent for chemical principles in the absence of the cardiac and lymphatic circulations. The reader will also find further experiments in support of our belief that a water-soluble substance, not in itself destructive of tissues, that has been injected at any point in the body of a frog cannot under normal conditions reach the cells of the central nervous system, completely

closed as it is in its bony encasement and well guarded by connective tissue elements at all intravertebral junctions and foramina, except by the usual route, namely, that of an intact cardiac circulation.

SUMMARY AND ADDENDA

We have presented many considerations and many facts in support of our belief that tetanus toxin and dye stuffs injected in an aqueous medium either intraneurally, subcutaneously, intramuscularly or intravenously are not carried in the axis cylinders, the lymphatic vessels, or the tissue spaces of peripheral motor nerves to the reacting cells of the central nervous system. We have also cited the recent investigations of anatomists who have traced the outflow of lymph from nerve trunks and have shown that it, like the lymph of other structures of the body, is added finally to the venous blood and not to the cerebrospinal fluid.

We furthermore called attention to a series of investigations that were carried out by Abel and Abel & Turner in the years 1910 to 1914, in which it was conclusively shown that alkaloids and dye stuffs cannot be distributed throughout the body by any peripheral mechanism, such as the "tissue spaces."

An account will be given in later papers of experiments that have been in progress in our laboratories for more than two years on the pathogeny of local tetanus, on the influence of complete denervation of muscles on the course of the poisoning, and on the reflex phenomena and other aspects of both experimental and natural tetanus. We find ourselves quite as unable to accept the current theories in regard to many of these characteristics as we are to accept the nerve transport theory, for the very good reason that this untenable theory is here also made to serve as the basis for their explanation.

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